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PSEUDOMONAS PYELONEPHRITIS IN THE NORMAL RAT AND IN THE NH_4CL-FED RAT

JEROLD CARL WOODHEAD

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PSEUDOMONAS PYELONEPHRITIS IN THE NORMAL RAT AND IN THE NH_4Cl-FED RAT

BY

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To My Parents and Andrea

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INTRODUCTION

The study of human and experimental pyelonephritis has greatly increased our knowledge of the disease process, but many fundamental questions remain unanswered. It is well known that the human disease is usually caused by autogenous E coli which gain access to the kidney by ascending the lower urinary tract. However, why the human kidney is susceptible to infection by E coli, while other organ systems are not, is unclear. Theories implicating renal blood flow, the intrarenal osmotic environment, and local inactivation of the host defense mechanisms involved in protecting against gram-negative bacterial infection, have been proposed to explain the susceptibility to infection, but none has been shown to be entirely valid. It is likely that multiple factors are involved.

One of the difficulties encountered in the study of pyelonephritis has been the inability to produce gram-negative bacterial pyelonephritis in most normal laboratory animals. The major exception is the mouse; and this species has poorly developed host-defense mechanisms against gram-negative organisms. Pyelonephritis in the rat has been produced for the most part only after some renal damage.

This paper will describe a model previously[21] demonstrated to enhance susceptibility to E coli pyelonephritis in the rat without evidence of structural damage to the kidney. It will be shown that this model also enhances the susceptibility of the rat to Pseudomonas pyelonephritis, and some discussion of the various factors thought to be involved in the action of this model will be presented. Finally, it will be shown that Pseudomonas is capable of producing renal infection in the normal rat.

REVIEW OF THE LITERATURE

Experimental Gram-Negative Pyelonephritis

The production of pyelonephritis with gram-negative organisms has proved to be extremely difficult in most normal laboratory animals. Although Gorrill[28,30] produced pyelonephritis in mice with a variety of gram-negative organisms as early as 1952, rats have proved to be highly resistant to such infection. The mouse has a poorly developed serum complement system, whereas this system is well developed in the rat[53]. Since gram-negative bacteria are generally susceptible to the complement-dependent bactericidal activity of serum[53], this difference provides a possible explanation for the vulnerability of the mouse kidney to coliform infection[24,28,30,31,35,36] and for the resistance of the rat kidney to such bacillary infection[10,21,30,32]. Gram-positive organisms, which are not affected by the complement-dependent bactericidal action[48], do cause pyelonephritis in the normal rat.

Gram-negative pyelonephritis has been produced, however, in rats whose kidneys have been scarred by previous staphylococcal infection[40], electrocautery[46], injection of sterile saline[20], and direct renal injection of bacteria[20]. Other mechanisms of renal injury such as transabdominal renal massage[11], chemical injury[47], renal vein occlusion[25], and ureteral ligation[22,32] also predispose to development of bacillary pyelonephritis in the rat. All of these procedures produce renal damage. By contrast, metabolic acidosis induced by NH_4Cl ingestion has been demonstrated to predispose to E. coli pyelonephritis[21], as has dehydration[6]; no histological damage was found. The recent demonstration by Fried[23] that a strain of hemolytic E. coli will produce pyelonephritis

in the normal rat is of considerable interest.

Medullary Vulnerability to Infection

It is now generally held that the renal papilla is the site where acute pyelonephritis is initiated. Papillary vulnerability to bacterial infection is well known. In 1956, de Navasquez[40] produced acute coliform pyelonephritis in rats whose kidneys had been scarred by a previous, healed, staphylococcal infection. Abscesses were strictly localized to the areas involved by the scars, but no mention was made concerning the specific location of these scars. As a result of these findings, Rocha _et al_[46] then demonstrated that lesions produced by electrocautery could also serve as foci for gram-negative abscess formation in the rat kidney. The important observation these authors made was that only the lesions in the medulla were associated with significant bacterial multiplication and abscess formation. It is thus apparent that blood borne bacteria localize and multiply in medullary lesions much more readily than they do in cortical lesions. The magnitude of this medullary vulnerability to infection was stressed by Freedman and Beeson[20], who showed that as few as 10 bacteria injected directly into the renal medulla produced pyelonephritis, whereas 10^5 bacteria are required to cause infection when injected into the cortex. Rocha _et al_[47] further substantiated the observations of medulla vulnerability by demonstrating that N_4-acetyl-sulfathiazole, which causes obstruction of collecting tubules by intraluminal crystallization, enhances the susceptibility of the rat to hematogenous bacillary pyelonephritis, while $HgCl_2$, which causes proximal tubular necrosis, does not. Renal vein occlusion[25] also increases susceptibility to infection. In all of the

above, factors such as intra-luminal hydronephrosis with urinary stasis, increased intra-renal tissue pressure and alteration of renal blood flow were probably involved in the production of pyelonephritis; but the intrinsic vulnerability of the medulla to infection was also believed to be a prime factor by the various authors.

The Course of Renal Infection

The question of how bacteria from the blood stream localize in the kidney has been studied by a number of groups. It is well known that the kidney does not possess a highly developed reticuloendothelial system as do the liver and spleen, and, consequently, must act as a mechanical filter. Guze and Beeson[32] studied the distribution and persistence of blood-borne E.coli in normal rats and showed that the absolute number of organisms deposited in the kidney was insignificant in comparison with the numbers in the liver, spleen and lungs. However, bacteria in the kidney persisted up to seven days with neither a tendency to multiply nor to be destroyed; the liver, spleen and lungs rapidly cleared the much larger bacterial implant. Thus, while the kidneys do not have a large role in the initial clearing of bacteria from the circulation, they demonstrate an inability to dispose of those bacteria which are trapped in the course of a bacteremia. Paplanus[41] postulated that this selective retention of bacteria without multiplication occurs because the medulla of the rat cannot efficiently clear a bacterial implant. The specific cause of this, however, he left unanswered.

The earliest studies on the course of hematogenous pyelonephritis were done by Gorrill[28] in 1952. He produced gram-negative pyelonephritis

in normal mice with Pseudomonas, and noted that the infecting dose had to be close to the dose at which 100 per cent mortality occurred in order to obtain high infection rates. The earliest detectable lesions occurred at 18-20 hours after injection, at which time "embolic focal nephritis" was demonstrable. This progressed to a focal nephritis with pus cells in the tubules and an interstitial inflammatory response around infected glomeruli. There was eventual progression to frank abscess formation at three days. Blood was sterile at 18 hours after injection, and the lungs by 36-48 hours. While liver and spleen initially removed greater numbers of bacteria from the blood because of their well developed reticuloendothelial systems, they were able to destroy the bacteria - unlike the kidney. Cultures of the liver and spleen at the time of death were positive, but the number of bacteria in these organs was much lower than the number in the kidneys.

Monma[39] demonstrated that although the kidney mechanically sieves bacteria from the blood, bacteria do not pass from the blood into the urine. When rabbits were intravenously challenged with several different bacterial species, urine cultures remained sterile during the seven hours after injection. During this period blood cultures were consistently positive. Guze et al[33] confirmed these observations with a study of non-obstructed enterococcal pyelonephritis in rats. They showed that the liver and spleen remove approximately 10^3 more bacteria from the blood than does the kidney, but that, whereas clearing occurs rapidly in the former two organs, rapid multiplication takes place in the latter. Bacteriuria was not detectable until three days after injection - at which time the earliest lesions of pyelonephritis were detectable histologically in the medulla. Progression to frank abscesses took place over the next few days.

It is of interest that while the earliest detectable bacteria were in the cortex, these did not multiply; cortical predominance lasted for only the first 12 hours post-injection, after which time the medulla had consistently higher bacterial titres.

Sanford _et al_[49] further studied the course of hematogenous pyelonephritis in the rat using renal massage[11] to enhance susceptibility to coliform infection. Bacterial localization in the glomeruli was transient in this study; after 24 hours, bacteria were seen only in the interstitial areas - no bacteria were demonstrated in blood vessels or tubules. In contrast to Gorrill's work, no focal nephritis was observed. Bacteria appeared in the urine only after microabscesses developed in the parenchyma and ruptured through the tubular wall. Urinary infection, thus was felt to be a secondary process resulting from interstitial abscess formation, not from filtering of bacteria from blood to urine or from primary multiplication of bacteria within the tubules.

In all of these studies the medulla was the major site of bacterial localization and persistence following bacteremia. The inability of the medulla to clear a bacterial implant is undoubtedly of significance in the observed vulnerability of this region of the kidney to infection, but the reasons for the medullary deficiency are incompletely understood.

The Medullary Environment and Its Effect on Susceptibility to Infection

The medulla's unusual susceptibility to infection can be explained in part by its peculiar environment. The interstitial fluid of the medulla is persistently hypertonic, even during diuresis[12,37]. Andriole has presented evidence supporting the role of medullary hypertonicity as

a major factor in susceptibility to pyelonephritis[6]. Andriole and Epstein[8] showed that water diuresis, with consequent reduction of medullary osmolality towards, but not reaching, isotonicity with plasma, can prevent the development of staphylococcal pyelonephritis and significantly alter the course of previously existing pyelonephritis. The same effect of diuresis was demonstrated for Streptococcus faecalis[7]. Acquatella et al[2] have presented evidence which they interpret as showing the anti-bactericidal effect of hypertonic medullary interstitial fluid. They showed, in in vitro studies, that urine having an osmolality greater than 500 mOsm/kg significantly depressed the bactericidal action of serum. These authors postulate that when renal medullary interstitial fluid is hypertonic, the bactericidal activity of serum is severely reduced. They suggest that this anti-bactericidal effect might be mediated by interference with those defense mechanisms dependent on complement. Indeed, Wardlaw[53] has shown that complement fixation and immune hemolysis are inhibited in hypertonic environments.

The importance of the acute inflammatory response as a general host defense mechanism preventing bacterial multiplication has been stressed by Sheldon and Bauer[50] and Cohn[14-16]. Especially important in this response are the early influx of cells and their ability to actively phagocytize and destroy bacteria. Both migration of cells and phagocytosis of bacteria appear to be depressed in the renal medulla. Rocha and Fekety[45] showed that the inflammatory response in the medulla is quantitatively deficient and delayed in the normal rat. And Andriole[5] has presented evidence that diuresis enhances this response both quantitatively and temporally. Chernew and Braude[13], furthermore, have shown that the phagocytic activity of polymorphonuclear leukocytes is

markedly decreased in hypertonic urine, and that reduction of urine osmolality resulted in improved phagocytosis. Diuresis increases medullary blood flow[51]; and the relationship between medullary blood flow and urine concentration has been established[1,38]. Medullary hypertonicity may also be important by predisposing to persistence of protoplast forms of bacteria[3]; the decrease in osmolality which accompanies diuresis, however, is probably sufficient to lyse most L-forms.

Diuresis then, appears to protect against pyelonephritis in a number of experimental models by enhancing various renal defense mechanisms which may be impaired at the tonicity of the nondiuresed medulla. However, since the *in vivo* models which have been used to demonstrate the protective effect of diuresis have used gram-positive organisms, the inference that the same effect holds true for gram-negative organisms cannot be made. Indeed, in direct contrast to the findings with gram-positive organisms, Freedman[19] has demonstrated that diuresis predisposes to the development and persistence of coliform bacteriuria, when organisms are introduced into the bladder. Diuresed rats were unable to clear *E. coli* from the urinary tract, and as few as ten (10) organisms injected into the bladder persisted and multiplied, with seeding of the kidneys. By contrast, rats on a normal water intake can clear as many as 10^7 bacteria from the bladder urine within 3-4 days, without renal seeding. The production of hematogenous *E. coli* pyelonephritis, though, was not enhanced by diuresis. Danzig[18] confirmed Freedman's findings and further showed that diuresis enhances the development of pyelonephritis when *E. coli* are injected directly into the renal medulla. Sterile medullary lesions were also more frequently the site of abscess

formation in diuresed than non-diuresed rats given intra-bladder inocula of coliform bacteria. The same phenomenon has been demonstrated in mice by Keane[35], Keane and Freedman[36], and Furtado and Freedman[24]. Thus, while there is much evidence to indicate that the hyperosmolality of the renal medulla has an important anti-bactericidal action, there are obviously factors other that hyperosmolality which affect the host response to renal infection.

The Renal Medulla and the Complement System

In 1959, Beeson and Rowley[10] demonstrated that the kidney exerts a local anti-complementary effect on serum which they felt was responsible for the peculiar susceptibility of renal tissue to infection by coliform bacteria. The anti-complementary effect was shown to parallel the glutaminase activity of the renal tissue studied, with the fourth component of complement (C'4) being inactivated. They were unable to demonstrate any difference in the anti-complementary action of cortical and medullary tissue: the factor involved in the effect was localized to the renal tubular system as a whole.

Freedman and Beeson[21] studied rats made metabolically acidotic by the chronic ingestion of NH_4Cl and demonstrated an increased susceptibility to renal infection by a strain of E. coli which is nonpathogenic in normal rats. The increased susceptibility was directly related to the renal glutaminase activity, which, in turn, was directly related to the duration of NH_4Cl ingestion. Weight loss was excluded as a contributing factor, and there were no detectable histological changes associated with chronic NH_4Cl ingestion. Neutralization of the acidosis by the substitution of $NaHCO_3$ for NH_4Cl resulted in a prompt decrease

in the renal glutaminase activity and a decrease in the rate of renal infection. Significantly, other organs - spleen, liver and lung - did not demonstrate an increased infection rate with acidosis. An intrarenal factor was postulated by these authors as being responsible for the increased susceptibility to infection, and the association of the renal ammonia producing system with this enhanced susceptibility supports the hypothesis of Beeson and Rowley that an anti-complementary action is involved.

The renal response to acidosis and in particular to the acidosis produced by NH_4Cl ingestion has been covered fully in excellent review articles by Pitts[42,43] and in textbooks of renal physiology[44]. The precise mechanisms involved in the production of ammonia by the kidney in the face of metabolic acidosis are still somewhat unclear[4,9,17,26,27,34], but the basic mechanism of tubular epithelial cell production of ammonia demonstrated in 1940, by Walker[52] is still generally accepted. Pitts, in his latest review[43], states that the two enzyme systems of major importance in the de novo synthesis of ammonia in the kidney are Glutaminase I and Glutamate dyhydrogenase. These enzymes account for approximately 90 per cent of renal ammonia production. The former of the two enzymes was the one found by Beeson and Rowley[10] to be most closely associated with the anti-complementary action of renal tissue.

It is evident from this brief review of the literature of experimental pyelonephritis that there are clearly several important variables in the different experimental systems. These include the species of laboratory animal, route of infection, bacterial species, and whether the kidney is normal or damaged. The medulla is much more vulnerable to infection than the cortex, but the roles of interstitial osmolality and of medullary anti-complementary action are still unclear.

THE SCOPE OF THE EXPERIMENTAL WORK

The experiments to be reported in this paper were devised to further explore the observations made by Freedman and Beeson[21] that rats made chronically acidotic by NH_4Cl ingestion demonstrate an increased susceptibility to E. coli pyelonephritis. An anti-complementary action of the ammonia produced by the kidney in response to acidosis was the mechanism originally postulated to explain this enhanced susceptibility. However, Acquatella et al[2] and Andriole[6] have suggested that the enhanced susceptibility to coliform pyelonephritis can be explained by the increased medullary tonicity which accompanies chronic NH_4Cl ingestion. Hence, it was felt to be important to further investigate the response of the kidney to gram-negative bacterial challenge during chronic NH_4Cl acidosis, with particular emphasis on the relation between urinary - hence medullary - tonicity and susceptibility to infection.

The organism chosen for study was Pseudomonas aeruginosa, since this is an increasingly important urinary pathogen among chronically ill and debilitated patients and in those whose normal body defense mechanisms have been depressed by various forms of therapy. It was of interest to determine whether NH_4Cl ingestion would enhance the susceptibility of the rat to renal infection by gram-negative organisms other than E. coli. Study of the infectivity of an opportunistic pathogen such as Pseudomonas in a model which involves impaired host defense mechanisms was felt to be worthwhile.

Experiments were designed using five groups of animals: Group I (Normal rats) given water and chow pellets ad lib; Group II (NH_4Cl) allowed free access to chow pellets and a 1.6% solution of NH_4Cl;

Group III (NH_4Ac) paired with NH_4Cl rats for consumption of chow pellets and a 2.3% solution of $NH_4C_2H_3O_2(NH_4Ac)$ - equimolar with the NH_4Cl solution; Group IV (Food Restricted) limited in food consumption and allowed water ad lib; and Group V (Water Restricted) with restricted water intake and given ad lib chow pellets. Each of the groups was challenged with a standard dose of Pseudomonas intravenously and then evaluated for rates of infection and mortality, and the possible influence of such factors as urine pH,urine specific gravity, and weight loss on the development of renal infection.

MATERIALS AND METHODS

Animals

White, male, Sprague-Dowley rats ranging in weight from 150-450 grams (most between 200-350 grams).

Bacterial Strain

The strain of Pseudomonas aeruginosa used in these experiments was isolated from a patient at the Yale-New Haven Hospital and has been previously described (24).

To obtain a culture containing between 1-9 x 10^8 viable bacteria per ml., nutrient broth was inoculated from the stock culture and allowed to remain overnight in the 37° C incubator. An aliquot of 0.5 - 1.0 ml. from this overnight culture was pipetted into fresh nutrient broth and incubated at 37° C for 4-5 hours. The standard inoculum consisted of 0.5 ml. of the undiluted 4-5 hour culture and was injected via a lateral tail vein.

Experimental Plan

Rats were placed on appropriate food and fluid regimens for a period of 10-14 days. Details will be described for each individual experiment. Intravenous challenge with the standard inoculum was then performed. Sacrifice occurred on the fourth day after challenge, at which time urine, heart blood and kidneys were examined. Details of bacteriological studies are described below. Rats dying before sacrifice were noted, but no autopsies were done.

Weight was recorded at the beginning of each experiment, prior to injection and at sacrifice. Urine specific gravity was determined on a spot urine sample prior to injection and on the bladder aspirate

at sacrifice. The spot urine was obtained by inducing a rat to void into a clean petri dish. Food and fluid consumption were measured each day where appropriate, and were replenished as needed. In the majority of cases the animals were housed singly, but because of limited space in the animal room it was necessary to house rats two to a cage in some experiments. The food and fluid intake in these latter cases was determined as the average of the total consumption for the two rats.

Urine specific gravity rather than osmolality was measured for a variety of reasons. The spot urine collection method was generally not productive of sufficient quantities of urine for osmolality determination. Total urine output was not measured because of the inability to prevent contamination of the urine by the watery-pasty feces excreted by the NH_4Cl-fed rats. A comparison was made early in the course of the experimental work between the specific gravity and osmolality of a number of urine samples, and on the basis of this comparison it was felt to be justifiable to measure only specific gravity on all urine samples.

Bacteriological Studies

Under intra-peritoneal pentobarbital anesthesia, the abdominal wall was cleansed with Zephiran ® and alcohol and the abdominal cavity was opened. The organs to be examined were removed and placed in sterile petri dishes from which they were later transferred to sterile tissue homoginizers after having been examined for abscesses. Whole kidneys were ground in 9.0 ml. of physiological saline, resulting in a dilution of approximately 10^{-1}. Serial ten-fold dilutions were made of the homogenates and plated on the surface of solid agar. After incubation

at 37° C for 24-48 hours, colony counts were made. Urine for culture was obtained at the time of sacrifice by needle aspiration of the urinary bladder and was plated onto solid agar using a calibrated loop containing 0.001 ml. Urine was not cultured before injection of bacteria. Heart blood for culture was obtained by direct trans-thoracic cardiac puncture at sacrifice; 0.1 ml. was plated onto solid agar. Grossly contaminated cultures served to exclude the animal from consideration. However, in the case of single, isolated contaminated cultures, where the contamination was clearly introduced during the bacteriological studies, the particular animal was not excluded from the study. While the contaminated culture was not considered in the experimental results, in each case the presence of a significant infection was clearly ascertainable from the non-contaminated cultures for the animal in question.

Criteria of Infection

Significant infection of kidneys was considered to have occurred when more than 5×10^4 viable bacteria could be grown from the organ OR when a gross abscess was present with any number of viable bacteria cultured from the kidney. In the majority, an abscess was associated with greater than 5×10^4 viable bacteria. Urine was considered infected when 10 more colonies could be grown from the sample - equivalent to more than 10^3 bacteria per ml. of urine. All positive plates were identified as containing Pseudomonas by colony morphology, characteristic odor and Gram stain. Standard fermentive techniques were used where necessary to identify organisms recovered from culture.

Diet

All rats were fed Purina Lab Chow Pellets, as specified below.

Drinking Solutions

All solutions were made with tap water. Solutions of 1.6% (wt/vol) NH_4Cl; 2.3% (wt/vol) $NH_4C_2H_3O_2$; and tap water were used in all the experiments. Rats were allowed access to these solutions as specified for each experiment.

Miscellaneous

pH was determined with nitrazine paper; specific gravity of urine with an optical densitometer.

RESULTS

Determination of Infection and Mortality Rates

The infection rate is expressed as the number of rats with infection, as defined above, divided by the number of rats alive 4 days after infection at the time of sacrifice i.e. % infection = (# infected/# alive at sacrifice) x 100. Thus, rats dying prior to sacrifice are not considered in the infection rate. This procedure was followed because the majority of deaths occurred within 48 hours after bacterial challenge and were assumed to have been caused by septicemia. Gorrill[31] observed that mice dying within this period after infection did not show renal lesions of pyelonephritis. Because of the similarities of the infection process in the mouse and rat, it seemed likely that this would also be true in rats. In these experiments, three rats died approximately 72 hours after bacterial challenge, and one of these was autopsied and found to have renal abscesses. Because autopsies were not routinely performed on rats dying after bacterial challenge, however, the cause of death of rats dying 1-3 days after challenge is unknown. The three rats which died at 72 hours were not considered in the calculation of infection rate.

Mortality rate was defined as the number of rats dying after bacterial challenge, at any interval prior to sacrifice, divided by the number of rats receiving the inoculum i.e. % Mortality = (#Dead/# receiving inoculum) x 100. The mortality, like the infection rate, was found to be dose dependent (see Table I, Figure I).

TABLE I

Mortality and Infection as Related to Inoculum Size

Inoculum		$1\text{-}9 \times 10^8$/ml					$<10^8$/ml					$>10^9$/ml				
			Dead		Infected"			Dead		Infected"			Dead		Infected"	
Group¢	N*	n**	#	%	#	%	n**	#	%	#	%	n**	#	%	#	%
I Normal	27	15	4	27	2	18	8	0	0	0	0	4	3	75	1	100
II NH_4Cl	59	38	4	11	20	59	12	1	8	1	9	9	8	89	1	100
III NH_4Ac	18	12	3	25	3	33	-	-	-	-	-	6	6	100	-	-
IV Food Restr.	26	26	5	19	5	24	-	-	-	-	-	-	-	-	-	-
V Water Restr.	22	10	1	10	3	33	-	-	-	-	-	12	0	0	5	42

* N = Total number of rats in each group

** n = Number of rats in each sub-group

¢ For details see individual results section

" For method of calculation of the infection rate see text

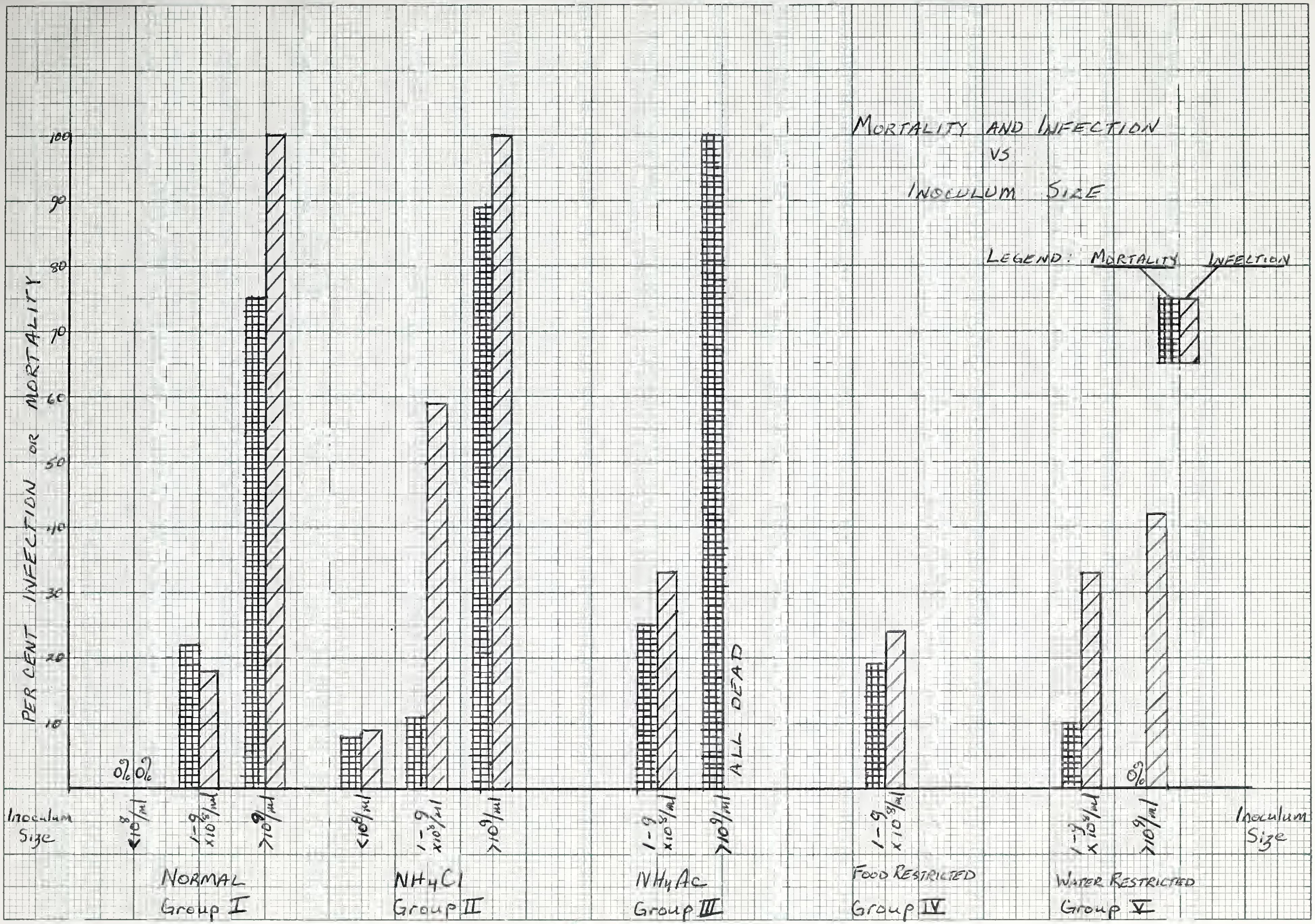

MORTALITY AND INFECTION
VS
INOCULUM SIZE
LEGEND: MORTALITY INFECTION
PER CENT INFECTION OR MORTALITY
100
90
80
70
60
50
40
30
20
10
0% 0%
ALL DEAD
0%
Inoculum Size
<10^8/ml
1-9 x10^8/ml
>10^9/ml
<10^8/ml
1-9 x10^8/ml
>10^9/ml
1-9 x10^8/ml
>10^9/ml
1-9 x10^8/ml
1-9 x10^8/ml
>10^9/ml
Inoculum Size
NORMAL
Group I
NH4Cl
Group II
NH4Ac
Group III
FOOD RESTRICTED
Group IV
WATER RESTRICTED
Group V

FIGURE 1

Inoculum Size

The size of the inoculum proved to be a significant variable in the induction of Pseudomonas peylonephritis. Becuase of the variable growth of Pseudomonas in culture, the size of the inoculum was difficult to control. Hence in some experiments, rats received numbers of organisms which were either inadequate to produce infection or highly lethal. Table I and Figure I summarize the experimental date pertaining to inoculum size. The results show that an inoculum in the range from 1-9 x 10^8 bacteria per ml. was maximally infective and minimally lethal; less than 10^8 bacteria per ml. ($<10^8$/ml) uniformly failed to produce infection; and an inoculum of greater than 10^9 bacteria per ml. ($>10^9$/ml) was highly lethal. The overall mortality in the rats receiving the high dose inoculum ($>10^9$/ml) was 55%. However, if the Group V (water restricted) rats are excluded, since they appeared to be unusually resistant to this high dose of bacteria, the mortality for the rats receiving the $>10^9$/ml inoculum is 90%. These data indicate that the dose range over which the strain of Pseudomonas used in these experiments was infective without excessive mortality was narrow and was extremely close to the dose at which mortality approached 100 per cent. Unless otherwise specified, the results below include only experiments in which rats received an inoculum in the range of 1-9 x 10^8/ml.

Specific Variables Studied

In all experiments the fluid consumption of the rats, weight change, and urinary pH and specific gravity were determined, both before bacterial challenge and during the period between challenge and sacrifice. Table II summarizes the data on these parameters for

the rats receiving the 1-9 x 10^8/ml inoculum, and Figure II compares them with the infection rate for each experimental group. The only significant difference in any of the parameters between the two periods studied was fluid consumption; in every instance, except for the water restricted group, fluid intake decreased after bacterial challenge. In the water restricted group the intake increased because they were allowed access to more water, as will be discussed below. The decreased fluid intake which followed bacterial challenge is in accord with similar observations in mice made by Furtado and Freedman[24]. Food intake was ad lib in most cases, but when measured, a similar decreased consumption was noted after challenge. It is assumed that decreased fluid and food intake are a result of stress imposed by the infection, possibly an effect of bacterial endotoxin.

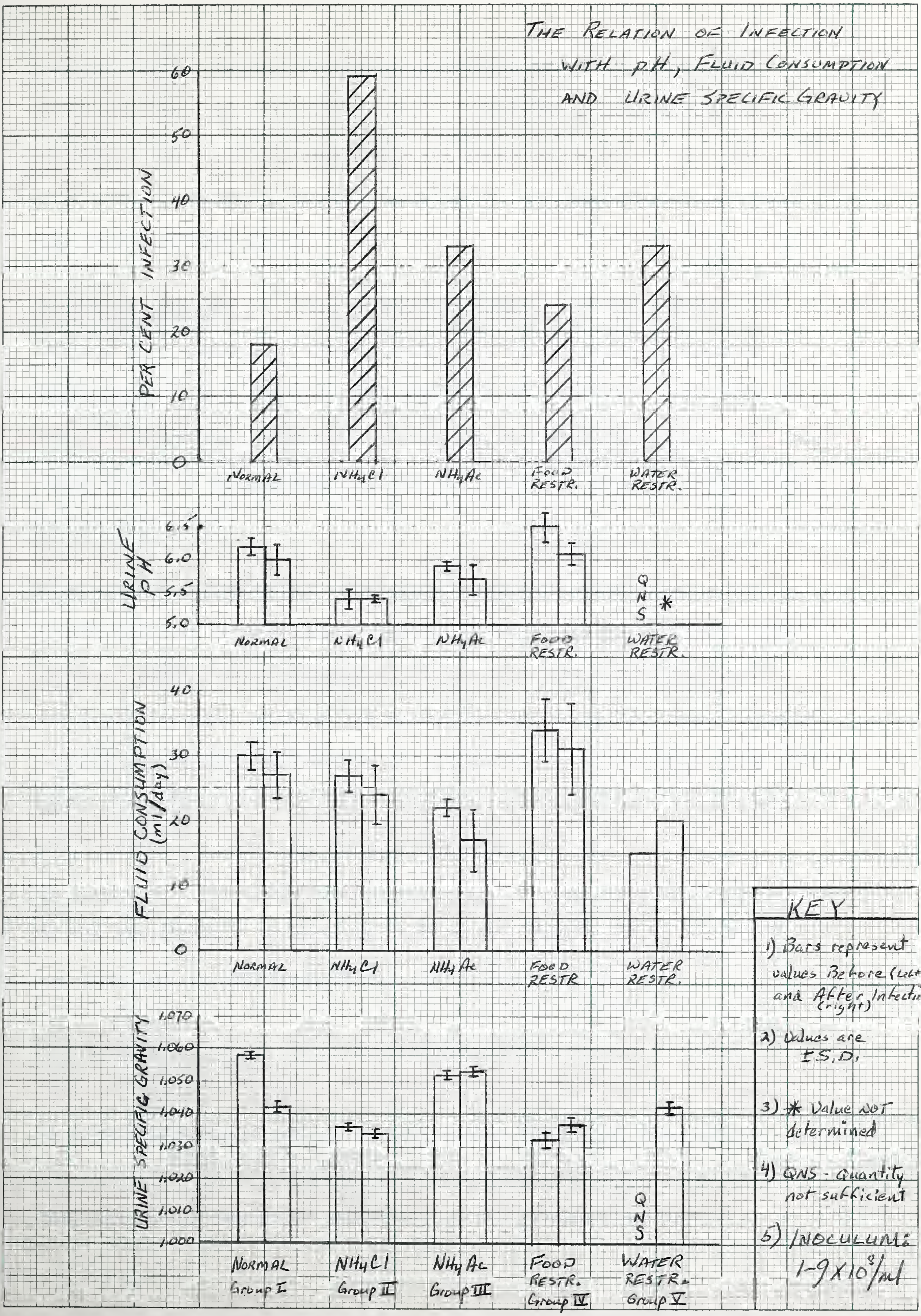
As seen in Table II, the animals all lost weight after bacterial challenge. This probably reflects in part the decreased fluid and food intake. Only certain groups of rats lost weight before infection, and the relation of this weight loss to infection will be discussed below.

Specific gravity and pH of the urine did not change much between the period preceding injection and that following; and the small changes which were seen were not significant ($p > .05$). Hence, only those values for the period prior to bacterial challenge will be considered in detail.

Effect of Diet and Fluid Alteration on Infection and Mortality Rates

The incidence of Pseudomonas pyelonephritis was studied in normal rats and in rats made acidotic by the chronic ingestion of NH_4Cl. To

FIGURE II



THE RELATION OF INFECTION WITH pH, FLUID CONSUMPTION AND URINE SPECIFIC GRAVITY

KEY

1) Bars represent values Before (Left) and After Infection (right)

2) Values are ± S.D.

3) * Value NOT determined

4) QNS - Quantity not sufficient

5) INOCULUM: 1-9 × 10³/ml

control for the osmolality factor associated with NH_4Cl ingestion, other rats were given equimolar NH_4Ac. Further, to evaluate the effects of medullary hypertonicity and weight loss, groups of rats were either dehydrated or food restricted. The experimental procedure has been described above.

Normal rats (Group I): Rats given free access to water and chow pellets were challenged intravenously with the standard inoculum of Pseudomonas (1-9 x 10^8/ml). The infection rate observed (see Table I) was 18%, while the mortality was 27%. It is thus apparent that the particular strain of Pseudomonas used in this work will cause pyelonephritis in the normal rat.

NH_4Cl-fed rats (Group II): A group of rats was allowed free access to a 1.6% solution (approximately 300 mM) of NH_4Cl and chow pellets, and then challenged with Pseudomonas. There was a 59% infection rate in the NH_4Cl-fed rats, with an 11% mortality (see Table I). These results indicate a definite enhancement of susceptibility in the rats given NH_4Cl ($p < .001$ by Chi Squares). The specific reason for this enhancement, however, is unclear, because the NH_4Cl-fed rats lost weight in contrast to the normal rats which gained weight over the course of the experiment. In addition, the osmotic load added by the NH_4Cl may have been an important factor, as in discussed below.

NH_4Ac-fed rats (Group III): A solution of NH_4Ac equimolar to the NH_4Cl given to the Group II rats was given to a third group of rats to investigate the effect of osmotic load. These rats were paired for both food and fluid intake with rats drinking NH_4Cl. Food was restricted to the number of pellets eaten the previous day by the

NH_4Cl-fed pair, and fluid was limited to the amount consumed the previous day by the NH_4Cl-fed rat. On the first day, the NH_4Ac-fed rats received one (1) chow pellet and 15 ml. of fluid.

The infection rate for the NH_4Ac rats was 33 per cent (3/9) with a mortality of 25 per cent (3/12) (see Table I). This infection rate is higher than that for the normal rats, but not as high as for those fed NH_4Cl. Some factors to be considered (as shown in Table II) include differences in fluid intake, a food consumption of 3 chow pellets per day compared with 4 per day for the NH_4Cl-fed group, and weight change. Also, the urinary specific gravity for the NH_4Ac-fed rats was 1.052, compared with specific gravity of 1.036 for the NH_4Cl-fed rats. Although the NH_4Ac-fed rats had more concentrated urine than the NH_4Cl-fed group, they were not dehydrated, despite a fluid intake of only 22 ml. per day, since the value of urinary specific gravity was within the range observed for the normal rats.

Food restricted rats (Group IV): Because of the weight loss associated with NH_4Cl ingestion, a study of the effects of inanition on susceptibility to infection was done. Three groups of rats were used, all of which were allowed free access to water: One group was restricted to one (1) chow pellet per day; a second group was restricted to two (2) chow pellets per day; and a third was paired with NH_4Cl-fed rats for food consumption. This latter group had a mean intake of three (3) pellets per day. Since specific gravity and infection rates are similar for the latter two groups, the results are combined. The data are tabulated in Table III. As can be seen, the infection rate for the severely food restricted group (1 pellet/day) was 33%; the rate for the other group was 20%.

TABLE III

Infection and Weight Loss in Food-Restricted Rats (Group IV)

Weight Loss	< 10%						> 10%					
		Dead		Infected		Specific		Dead		Infected		Specific
Food Intake	n	#	%	#	%	Gravity	n	#	%	#	%	Gravity
1 Pellet/day	0	-	-	-	-	-	10	5	45	2	33	1.050
2-3 Pellets/day	15	0	0	3	20	1.026	1	0	0	0	0	1.010

Inoculum: 1-9 x 10^8/ml.

The obvious differences between the one-pellet/day rats and those consuming 2 or 3 per day are weight loss and urine specific gravity. The former group demonstrated severe weight loss, with all rats having greater than 10% loss of body weight; in the latter group all animals but one had less than 10% weight loss. The mean urinary specific gravity for these groups was 1.050 and 1.026, respectively.

It appears that only when inanition is severe will infection and mortality be enhanced, and mortality more so than infection. The increase in susceptibility observed with severe weight loss was less than that observed in NH_4Cl-fed rats, (33% infection rate compared to 59%) although this difference is of questionable significance because of the small number of rats involved.

Water restricted rats (Group V): To investigate the effect of dehydration, and hence of medullary hypertonicity, on susceptibility to Pseudomonas pyelonephritis, a group of rats had its water intake restricted but was given chow pellets ad lib. The infection rate was 33 per cent, and the mortality was 10 per cent (see Table I). These rats were started on a water limitation of 15 ml. per day, but this was increased to 20 ml. per day at the time of bacterial challenge because of excessive weight loss. The rats were obviously dehydrated at the time of challenge and no urine could be obtained from them.

Since these rats all had severe weight loss (>10%), it is possible that a factor related to weight loss rather than dehydration or medullary hypertonicity produced the enhancement to infection that was observed. The infection rate for Group V (33%) is the same as that of the food restricted rats (Group IV) with similar weight loss (see Table III). Because no urine could be obtained, any conclusion about the relation

of infection to a specific degree of urinary hypertonicity cannot be made.

It is of interest that in one experiment a group of water restricted rats received an inoculum of $>10^9$/ml. These rats were initially larger than those described above by about 100 grams, had less severe weight loss and were given 20 ml. water per day throughout the course of the experiment. The mean specific gravity of their urine was 1.072, and they were obviously dehydrated. The infection rate for these rats was 42 per cent, but there was no mortality. As was discussed previously, all other rats receiving the high dose inoculum showed mortality close to 100 per cent, with an infection rate in the survivors of 100 per cent (see Table I Figure I). The apparent resistence of the water restricted animals to the effects of the $>10^9$/ml inoculum is difficult to explain. Table IV compares the water restricted rats according to weight loss and infecting dose. It is evident that the infection rate for those receiving the higher dose inoculum is greater than that for the other rats. The numbers of rats in these groups makes statistical comparison with the NH_4Cl-fed group unproductive. However, it would appear that if dehydration (increased renal medullary tonicity) per se were the factor primarily involved in enhancement of susceptibility, the effect should have been manifest more conclusively with the $>10^9$/ml inoculum.

Infection and Urine pH

The relation between infection rate and urine pH in these experiments is shown in Figure II. The urine pH of the NH_4Cl-fed rats was acid with respect to the normal range of pH for the rat (pH 5.5-6.5), although not markedly so. With a mean pH of 5.4, the NH_4Cl-fed rats

TABLE IV

Infection and Weight Loss in Water-Restricted Rats (Group V)

Inoculum Size	1-9 x 10^8/ml		>10^9/ml	
Weight Loss	<10%	>10%	<10%	>10%
Number	1	9	12	0
Deaths	0	1	0	-
% Mortality	0	11	0	-
Infections	0	3	5	-
% Infected	0	38	42	-

can be contrasted with the NH_4Ac-fed group at pH 5.9, with the food restricted group at pH 6.5, and with the normal rats at pH 6.2. Urine samples were unobtainable from the water restricted rats. There does appear to be a correlation between low pH and high infection rate, but the data are of questionable significance.

Infection and Urine Specific Gravity

Figure II relates infection rate to the urinary specific gravity of each of the experimental groups, while Figure III shows the distribution of infection over the range of specific gravity for all the urines obtained. Specific gravity varied widely according to the fluid and food intake. The lowest average urinary specific gravity was that of the food restricted group (specific gravity 1.032). Next lowest were the urines from NH_4Cl-fed rats with a specific gravity of 1.036. The NH_4Ac-fed rats and the normal water rats had similar urine specific gravities (1.052 and 1.058, respectively). Urine was not obtainable from the water restricted group.

It is apparent that infection does not correlate with increasing specific gravity. In fact, the majority of the infections in all the groups occurred in rats with urine specific gravities between 1.025 and 1.040. These infections were mostly in NH_4Cl-fed rats. It would thus seem that medullary tonicity, as reflected by urinary specific gravity, is not the determining factor for the enhancement of susceptibility to *Pseudomonas* pyelonephritis observed in the NH_4Cl-fed rats.

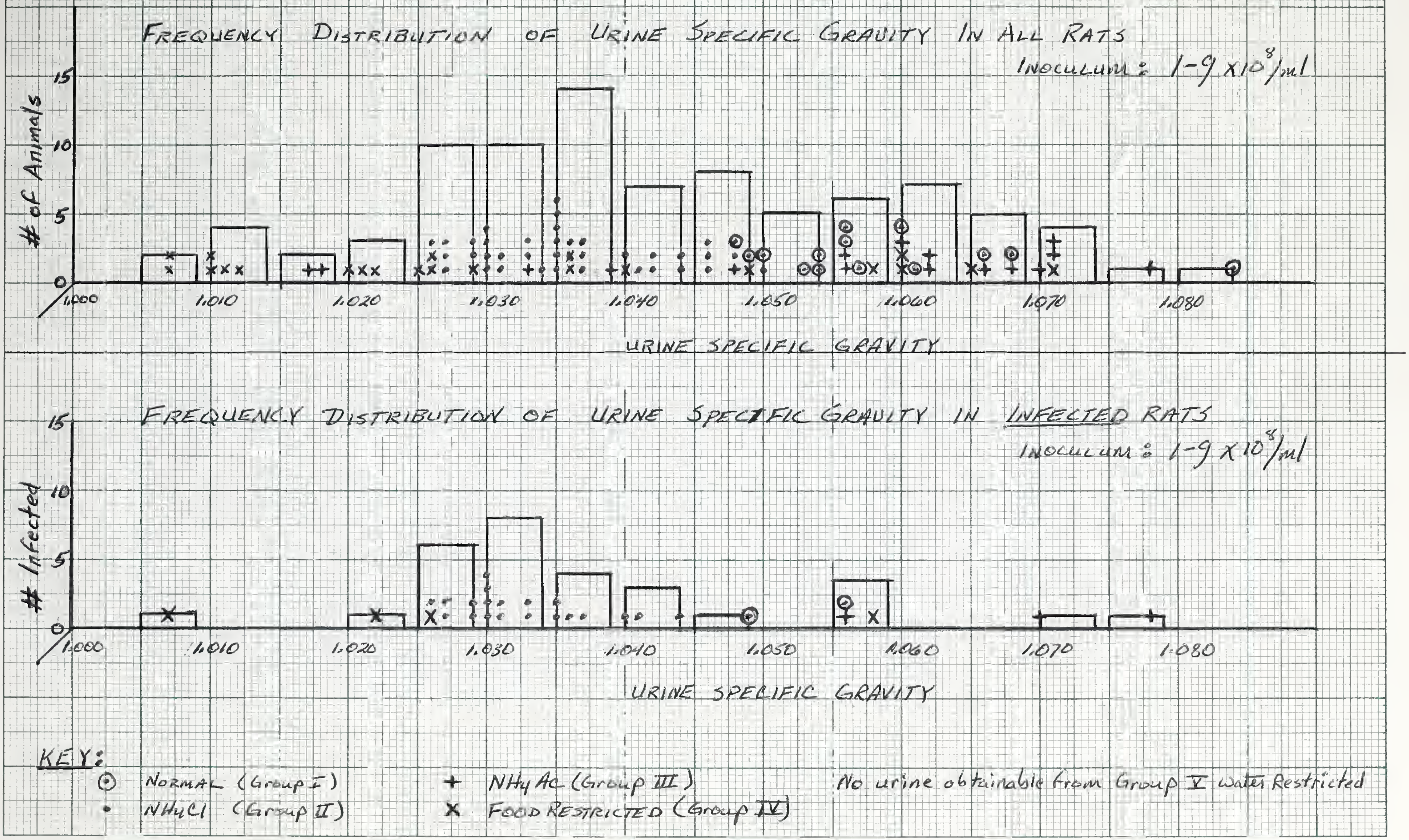

FREQUENCY DISTRIBUTION OF URINE SPECIFIC GRAVITY IN ALL RATS
INOCULUM: 1-9 X10⁸/ml
# of Animals
0
5
10
15
1.000
1.010
1.020
1.030
1.040
1.050
1.060
1.070
1.080
URINE SPECIFIC GRAVITY
FREQUENCY DISTRIBUTION OF URINE SPECIFIC GRAVITY IN INFECTED RATS
INOCULUM: 1-9 X10⁸/ml
# Infected
0
5
10
15
1.000
1.010
1.020
1.030
1.040
1.050
1.060
1.070
1.080
URINE SPECIFIC GRAVITY
KEY:
⊙ NORMAL (Group I)
• NH4Cl (Group II)
+ NH4 Ac (Group III)
X FOOD RESTRICTED (Group IV)
No urine obtainable from Group V water Restricted

FIGURE III

Infection and Weight Loss

To examine the relationship between weight loss and susceptibility to infection, the experimental groups were divided according to the severity of the weight loss observed. The data are summarized in Table V and shown graphically in Figure IV. On the left side of the table the average weight change is given for each group. No clear correlation between increased susceptibility and weight loss can be made. This is demonstrated by comparison of data for the NH_4Ac-fed rats (weight gain of +19 grams), the food-restricted rats (weight loss of -21 grams), and the water-restricted rats (weight loss of -44 grams); each of these groups has a comparable infection rate.

On the right side of Table V, the data have been broken down on the basis of the extent of weight loss ($<$or$>$10%). There is some suggestion that greater weight loss is associated with increased susceptibility to infection within each experimental group. For example, the NH_4Cl-fed rats with $<$10% weight loss have an infection rate of 52 per cent; thos with $>$10% weight loss have a rate of 78 per cent. Similarly, the food restricted rats showed infection rates of 20 per cent and 33 per cent for $<$10% and $>$10% weight loss, respectively. While severe weight loss does seem to enhance susceptibility to infection, it is apparently not the only factor operating to predispose to infection. It is obvious that the NH_4Cl-fed rats losing $>$10% body weight had a much higher infection rate than did water restricted rats with the same severe weight loss. Weight loss, then, appears to predispose to Pseudomonas pyelonephritis particularly when another contributing factor, such as NH_4Cl ingestion, is present.

TABLE V

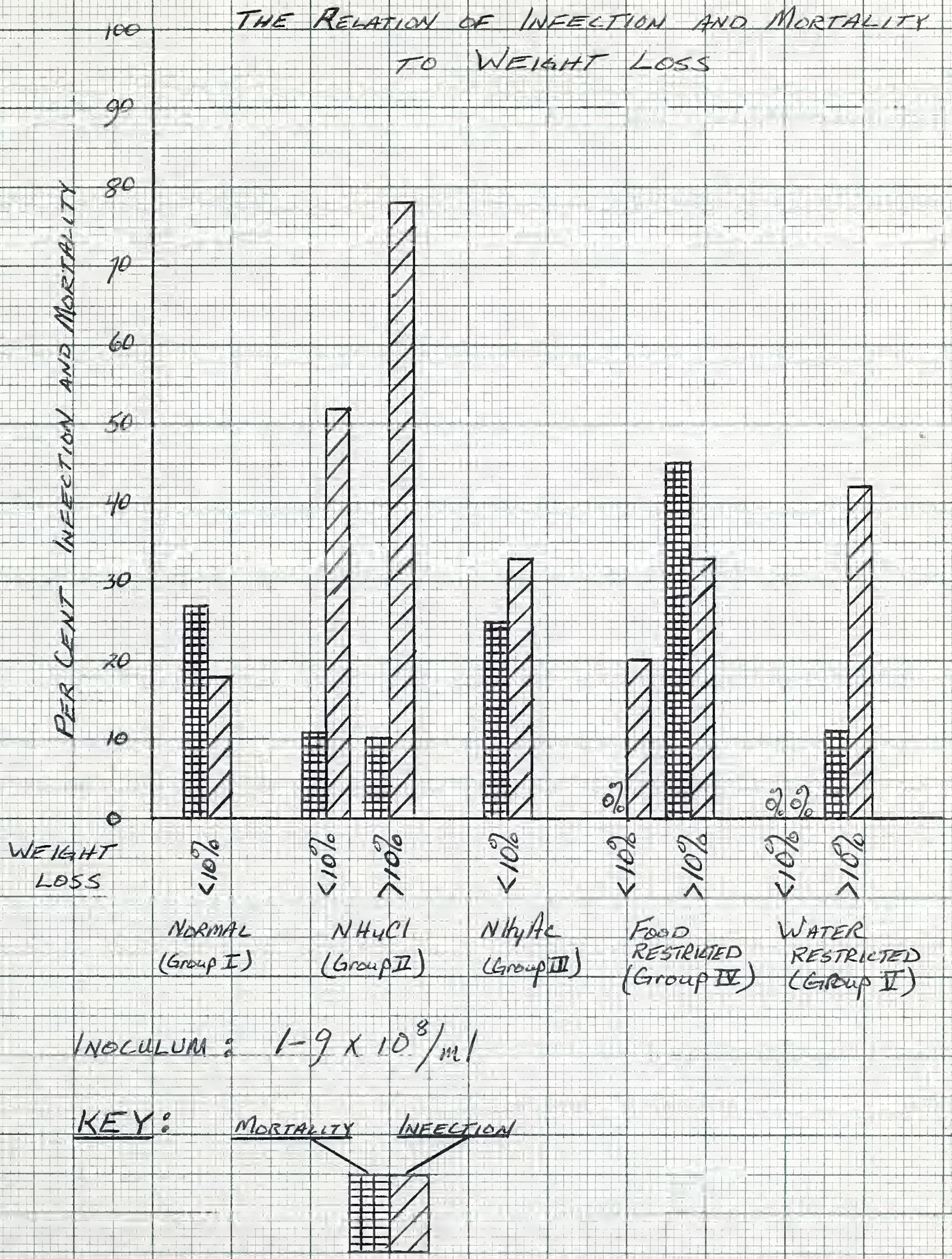
Relation Between Mortality, Infection and Weight Loss

							WEIGHT LOSS <10%						WEIGHT LOSS >10%					
Group¢	N*	Dead #	Dead %	Infected #	Infected %	Avg. Δ Weight (gram)	n**	Dead #	Dead %	Infected #	Infected %	Avg. Δ Weight (grams)	n**	Dead #	Dead %	Infected #	Infected %	Avg. Δ Weight (grams)
I Normal	15	4	27	2	18	+43	15	4	27	2	18	+43	-	-	-	-	-	-
II NH_4Cl	38	4	11	20	59	-6	28	3	11	13	52	+6	10	1	10	7	78	-40
III NH_4Ac	12	3	25	3	33	+19	12	3	25	3	33	+19	-	-	-	-	-	-
IV Food Restr.	26	5	19	5	24	-21	15	0	0	3	20	-1	11	5	45	2	33	-49
V Water Restr	10	1	10	3	33	-44	1	0	0	0	0	+49	9	1	11	3	38	-58

Inoculum: 1-9 x 10^8/ml

¢ For details see individual results section
* N = Total number of rats in each group
** n = Number of rats in each sub group

FIGURE IV



THE RELATION OF INFECTION AND MORTALITY TO WEIGHT LOSS

INOCULUM: 1-9 X 10^8/ml

DISCUSSION

These experiments were designed to investigate the response of the kidney to Pseudomonas challenge in the normal rat, in the rat made acidotic by NH_4Cl consumption, in the dehydrated rat, and in the starved rat. An attempt was made to correlate medullary tonicity, as reflected in urinary specific gravity, with susceptibility to infection. The results show that 1) Pseudomonas will produce pyelonephritis in the normal rat, with infection and mortality both directly dose-dependent; 2) NH_4Cl ingestion enhances the susceptibility of the rat to Pseudomonas pyelonephritis in a manner not obviously attributable to renal hypertonicity or to inanition; 3) Increased medullary tonicity produced by dehydration does not seem to greatly enhance susceptibility to infection by Pseudomonas; and 4) Starvation with severe weight loss enhances susceptibility to Pseudomonas infection, but appears to act mainly in a permissive manner, allowing another predisposing factor such as NH_4Cl ingestion to have a greater effect.

Pseudomonas Infection in the Rat

Pyelonephritis can be produced in the normal rat by intravenous challenge with Pseudomonas. The infection rate of 18 per cent is low, but is in keeping with the observation of Furtado and Freedman[24] that the strain of bacteria used produces pyelonephritis in approximately 34 per cent of normal mice. Since the vulnerability of the mouse to gram-negative pyelonephritis is well known to be greater than that of the rat, the lower infection rate observed here is not surprising.

The dose range within which pyelonephritis could be produced without excessive mortality was narrow, and close to the dose at which

mortality approached 100 per cent. These data are similar to those for mice obtained by Gorrill and de Navasquez using a different strain of Pseudomonas. Because the dose-response relationship is qualitatively identical in mice and rats, it can be postulated that the quantitative differences observed reflect the inherent vulnerability (or resistence) of the two species to infection by Pseudomonas. One of these factors could be the difference in the serum complement system observed in mice and rats.

NH_4Cl Ingestion and Pseudomonas Pyelonephritis

Because of the observation that Pseudomonas can produce pyelonephritis in the normal rat, it was felt that any factor which acted to inhibit the normal host defense mechanism should markedly enhance the susceptibility of the kidney to such infection. Previously, susceptibility to E. coli pyelonephritis was shown to be enhanced in rats fed NH_4Cl[21], and the inactivation of complement was proposed to explain the enhancement. The data presented in this paper support a similar enhancing action of NH_4Cl ingestion on susceptibility to Pseudomonas infection. The infection rate of 59 per cent in NH_4Cl rats contrasts strikingly with the 18 per cent infection rate observed for the normal rats.

Andriole[6] has postulated that NH_4Cl acts to enhance susceptibility to E. coli pyelonephritis by increasing renal medullary osmolality and thus providing a more favorable environment for the growth of bacteria. He does not feel that the anti-complementary action which has been demonstrated[10,21] during NH_4Cl acidosis is adequate to explain the observed change in vulnerability to infection; and he has shown that

dehydrated rats will develop E. coli pyelonephritis caused by a strain non-pathogenic in normal rats. However, in the experiments reported above, the enhanced susceptibility observed with NH_4Cl ingestion does not seem to be explained by increased medullary tonicity or inanition because infection rates in dehydrated and food-restricted rats were not comparable to that of the NH_4Cl-fed group. These two factors are considered in detail, below. A direct anti-complementary action may well be involved in producing this enhanced susceptibility to Pseudomonas pyelonephritis, although specific experiments to investigate this were not done.

Renal Hypertonicity and Pseudomonas Pyelonephritis

The data presented in these experiments with Pseudomonas do not seem to support Andriole's contention that dehydration is a major factor in increasing susceptibility to infection in rats drinking NH_4Cl. Rats dehydrated by the restriction of water to approximately one-half normal daily fluid intake did not demonstrate the same high infection rate observed in the NH_4Cl-fed rats. Given an inoculum in the range of 1-9 x 10^8/ml, the water restricted rats had an infection rate of 33 per cent, which, although greater than that for the normal rats, was less than the 59 per cent infection rate of the NH_4Cl-fed rats. The small number of rats in the water-restricted group precludes statistical significance of this observation. However, the fact that an inoculum of $>10^9$/ml given to a group of water-restricted rats increased the infection rate to only 42 per cent, with no mortality, lends credence to the conclusion that dehydration, with increased medullary tonicity cannot entirely explain the enhanced susceptibility of the NH_4Cl-fed rats.

It is of interest to note that the group of rats with the highest infection rate (NH_4Cl) had urinary tonicity lower than that of any other group except the food restricted group. This is in direct opposition to the observations of Andriole[6], who showed an increased urinary osmolality with NH_4Cl ingestion. It is doubtful that the specific gravity and osmolality values could be so markedly different without an absolute difference in urine concentration; and, indeed, a direct correlation between specific gravity alone was relied upon as a measure of urinary tonicity. It is noteworthy that there was a difference in fluid consumption for rats in the two experiments, although the NH_4Cl solutions were the same; Andriole's rats consumed an average of 20 ml/day, while the rats presented in this paper consumed an average of 27 ml/day. The latter value represents slightly less than the normal water consumption compared with normal. Thus, an absolute dehydration might account for the difference in urine concentration. Also, the group of NH_4Ac-fed rats drinking a solution equimolar with the NH_4Cl solution had urine specific gravities comparable to those of normal, water-drinking rats; The osmotic load did not cause a hypertonic urine to be produced. The infection rate observed for this group (33%) indicates that increased osmotic load alone cannot account for the enhancement seen with NH_4Cl.

Weight Loss and Pseudomonas Pyelonephritis

As has been mentioned, a group of rats was food deprived and allowed free access to water in an effort to determine the effect of weight loss on susceptibility to infection. Freedman and Beeson[21] had previously demonstrated a lack of correlation between loss and E. coli infection in all but the most severe degrees of inanition. The data presented

above demonstrate that weight loss *per se* does not predispose markedly to infection. However, when associated with other predisposing factors, such as NH_4Cl ingestion and possibly dehydration, weight loss does seem to contribute to enhanced susceptibility, especially when it is severe i.e. $>$10% of body weight.

SUMMARY

Experiments have been presented which demonstrate that:

1) Pseudomonas aeruginosa will produce pyelonephritis in normal rats, although at a low rate. The infection rate is dose dependent, with the optimal infecting dose being quite close to the dose at which mortality approaches 100 per cent.

2) NH_4Cl ingestion enhances the susceptibility of the rat to Pseudomonas pyelonephritis. This confirms previous studies with E. coli.

3) The enhanced susceptibility to infection in NH_4Cl-fed rats does not seem to be solely attributable to renal hypertonicity or weight loss.

4) Dehydration, with increased medullary tonicity, does not alone seem to markedly enhance susceptibility to infection.

5) Weight loss alone also does not markedly predispose to infection. However, when severe it may contribute to increased susceptibility to infection when another predisposing factor, such as NH_4Cl ingestion, is present.

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